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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: Post FOCUS Review of Ecotoxicity Studies for [redacted]

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[redacted]

This memorandum provides a review of nine ecotoxicity studies (2 studies on PMNs and 7 studies on analogous chemicals) in March 3, 2014 by Shell Chemical LP (sponsor). EPA has reviewed the submitted studies for 1) appropriateness of analogs, and 2) adequacy of studies for [redacted] In addition, EPA has revisited the test recommendation based on other pertinent analogous chemicals that EPA has located. The modified ecotoxicity profile table is provided in the section below (Table 2).

Background

[redacted]

Table 1. Case Number: [redacted] Studies submitted under [redacted]

Case Number Chemical Name	Typical Composition (%)	CAS RN	Trade Name
[redacted]	C8-9 C9-27.8 C10-46.3 C11-14.6	[redacted]	Shell GTL Solvent GS160
	C9-6.7 C10-28.9 C11-50.6 C12-12.6	[redacted]	Shell GTL Solvent GS170
	C9-8 C10-21 C11-32.4 C12-26.6 C13-8.8	[redacted]	Shell GTL Solvent GS210
	C10-5.7 C11-27.8 C12-46.7 C13-18.4	[redacted]	Shell GTL Solvent GS190
	C12-14.3 C13-36.6 C14-38.2 C15-8.3	[redacted]	Shell GTL Solvent GS215
	C14-24.4 C15-53.6 C16-19.0	[redacted]	Shell GTL Solvent GS250

[redacted]

Summary and Conclusions

The sponsor submitted nine ecotoxicity studies (2 studies on PMNs and 7 studies on analogous chemicals) in March 3, 2014. EPA has reviewed the submitted studies for 1) appropriateness of analogs, and 2) adequacy of studies with the following conclusions.

EPA reviewed the studies on the 2 PMN cases, provided by the sponsor, and has concluded that they are inadequate for the purposes of this PMN review, for the following reasons: 1) The substances tested were of unknown composition - the test

substances' purity is not provided and they are described as complex mixtures, 2) the studies used the Water Accommodated Fraction (WAF) method using nominal loading rates with only information of that "at 0 and 72 hours showed measured test concentrations of less than the limit of quantification" – it is not known what chemicals and quantities were actually tested in the WAF testing system.

EPA reviewed the studies on the analogous chemicals, provided by the sponsor, and has concluded that they are inadequate for the purposes of this PMN review, for the following reasons: 1) the carbon ranges of C4-10, C8-26, C11- C24 or C9- C29- branched and linear are too wide to match any of PMN substances, and 2) the test substances' purity is not provided, therefore, the test substance compositions are unknown.

All studies submitted by the sponsor on PMNs and analogous chemicals are considered to be inadequate by EPA. Although these studies are considered inadequate, the studies summaries are attached in Appendices 1 and 2 for information purposes only.

Moreover, EPA has located other pertinent analogous chemicals. Based on this additional information, the modified ecotoxicity profile table is provided in the section below (Table 2).

Based on the data from analog chemicals and typical chemical compositions of PMNs, EPA is modifying the test recommendation previously proposed from FOCUS. EPA recommends that the ecotoxicity chronic base set tests: Daphnid chronic toxicity test (OPPTS Test Guideline 850.1300), Fish early-life stage toxicity test (OPPTS Test Guideline 850.1400), and Algal toxicity test (OCSPP Test Guidelines 850.4500) are to be performed on [redacted]

Table 2. Ecotoxicity Profile of [redacted] - using analogous chemicals by EPA

	Fish 96-h EC_{50} (mg/L)	Aquatic Invertebrates 48-h EC_{50} (mg/L)	Aquatic Plants 96-h EC_{50} (mg/L) Biomass Growth Rate	Chronic Toxicity to Aquatic Invertebrates ChV (mg/L)	Chronic Toxicity to Fish ChV (mg/L)
[redacted]	**6.6 (m)**	**4.4 (m)**	**4.6** **>5.5**		-
[redacted]		**0.2 (m)**			-
[redacted]	18.4 (n) **0.11(m)**				-
[redacted]		**0.3 (m)** **0.4 (m)**			-
[redacted]		**0.2 (m)**			-
[redacted]				**0.024**	
[redacted]	0.11- 6.6 (RA)	0.011- 4.4 (RA)	>0.0059 - 4.6 (RA) >0.0059 - >5.5 (RA)	0.024(RA)	
[redacted]	0.11- 6.6 (RA)	0.011- 4.4 (RA)	>0.0059 - 4.6 (RA) >0.0059 - >5.5 (RA) **Rec Test**	0.0069 (RA) **Rec Test**	**Rec Test**
[redacted]	**>0.013 (m)**	**0.011 (m)**	**>0.0059 (72-h)** **>0.0059 (72-h)**	**NOEC=0.0057 (m)** **LOEC=0.0083 (m)** **0.0069 (ChV)**	-
[redacted]	0.11- 6.6 (RA)	0.011- 4.4 (RA)	>0.0059 - 4.6 (RA) >0.0059 - >5.5 (RA) **NEW TEST**	0.0069 (RA) **NEW TEST**	**NEW TEST**
[redacted] ar	0.11- 6.6 (RA)	0.011- 4.4 (RA)	>0.0059 - 4.6 (RA) >0.0059 - >5.5 (RA) **Rec Test** **NEW TEST**	0.0069 (RA) **Rec Test** **NEW TEST**	**Rec Test** **NEW TEST**
[redacted]	**NES**	**NES**	**NES**	**NES**	-
[redacted]	NES (RA)	NES (RA)	NES (RA)	NES (RA)	
[redacted]	NES (RA)	NES (RA)	NES (RA) **Rec Test**	NES (RA) **Rec Test**	**Rec Test**

- = no data; **Rec Test**= previously recommended tests from FOCUS;
NEW TEST = revised test recommendation from this review

A summary of analogous chemicals toxicity data provided in Table 2 is below.

Acute Toxicity to Fish

Alkenes, C6 [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/HigherOlefins.pdf
96-h LC50 =6.6 mg/L

Hydrocarbons, C7-C9, isoalkanes [redacted]
http://www.webnet.oecd.org/Hpv/UI/handler.axd?id=afd8ccb9-af39-43ca-b49c-5034972e-75dc
96-h LC50 =0.11 mg/L

n-Undecane [redacted]
http://www.epa.gov/chmrtk/hpvis/hazchar/Category_Higher%20Olefins_June_2011.pdf
96-h LC50 >0.013 mg/L

1-Tetradecene [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/AOalfaolefins.pdf
96-h LC50 = No effects at saturation

Acute Toxicity to Aquatic Invertebrates

Alkenes, C6 [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/HigherOlefins.pdf
48-h EC50 =4.4 mg/L

2.3.4-trimethylpentane [redacted]
http://www.webnet.oecd.org/Hpv/UI/handler.axd?id=afd8ccb9-af39-43ca-b49c-5034972e-75dc
48-h EC50 =0.2 mg/L

n-Octane [redacted]
http://www.webnet.oecd.org/Hpv/UI/handler.axd?id=afd8ccb9-af39-43ca-b49c-5034972e-75dc
48-h EC50 =0.3 mg/L
48-h EC50 =0.4 mg/L

n-Nonane [redacted]
http://www.webnet.oecd.org/Hpv/UI/handler.axd?id=afd8ccb9-af39-43ca-b49c-5034972e-75dc
48-h EC50 =0.2 mg/L

n-Undecane [redacted]
http://www.epa.gov/chmrtk/hpvis/hazchar/Category_Higher%20Olefins_June_2011.pdf
48-h EC50 =0.011 mg/L

1-Tetradecene [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/AOalfaolefins.pdf
96-h LC50 = No effects at saturation

Toxicity to Aquatic Plants

Alkenes, C6 [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/HigherOlefins.pdf
96-h EC50 =4.6 mg/L (Biomass)
96-h EC50 >5.5 mg/L (Growth rate)

n-Undecane [redacted]
http://www.epa.gov/chmrtk/hpvis/hazchar/Category_Higher%20Olefins_June_2011.pdf
72-h EC50 >0.0059 mg/L (Biomass, Growth rate)

1-Tetradecene [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/AOalfaolefins.pdf
96-h EC50 = No effects at saturation

Chronic Toxicity to Aquatic Invertebrates

1-Decene [redacted]
http://www.inchem.org/documents/sids/sids/HigherOlefins.pdf
21-d ChV = 0.024 mg/L

n-Undecane [redacted]
http://www.epa.gov/chmrtk/hpvis/hazchar/Category_Higher%20Olefins_June_2011.pdf
21-d NOEC= 0.0057mg/L
21-d LOEC=0.0083 mg/L
21-d ChV= 0.0069 mg/L

1-Tetradecene [redacted]
http://www.chem.unep.ch/irptc/sids/OECDSIDS/AOalfaolefins.pdf
21-d ChV = No effects at saturation

References

(1) SIDS Initial Assessment Report for SIAM 11 for Alfa Olefins. 2001. http://www.chem.unep.ch/irptc/sids/OECDSIDS/AOalfaolefins.pdf

(2) SIDS Initial Assessment Report for SIAM 19 for Higher Olefins. 2004. http://www.inchem.org/documents/sids/sids/HigherOlefins.pdf

(3) SIDS Initial Assessment Report for SIAM 30 for C7-C9 Aliphatic Hydrocarbon Solvents Category. April, 2010. http://www.webnet.oecd.org/Hpv/UI/handler.axd?id=afd8ccb9-af39-43ca-b49c-5034972e-75dc

(4) U.S. Environmental Protection Agency Hazard Characterization Document Higher Olefins Category June 2011 http://www.epa.gov/chmrtk/hpvis/hazchar/Category_Higher%20Olefins_June_2011.pdf

APPENDIX 1

The following studies on analogous chemicals are considered to be inadequate by EPA, and are included in this report for information purposes only.

EPA reviewed the studies below, provided by the sponsor, and has concluded that they are inadequate for the purposes of this PMN review, for the following reasons: 1) the carbon ranges of C4-10, C8-26, C11- C24 or C9- C29- branched and linear are too wide to match any of PMN substances, and 2) the test substances' purity is not provided, therefore, the test substance compositions are unknown.

Fish Ecotoxicity Tests:

Study 1:
The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.
Chemical Name: GTL Naphtha (Fischer-Tropsch), light, C4-10 - branched and linear
[redacted]

Harlan Laboratories Ltd conducted a 33-day early-life stage toxicity test in fathead minnow (*Pimephales promelas)* with an analog test substance (purity not provided; complex mixture) under semi-static conditions with daily renewal. This study was reported to follow OECD test guideline 210 "Fish, Early-Life Stage Toxicity Test" (1992), and OPPTS 850.1400. Based on the results of an acute toxicity test, two replicates of 30 eggs were exposed to the control (dechlorinated tap water) or to the test substance prepared as a Water Accommodated Fraction (WAF) at nominal loading rates of 1.0, 3.2, 10, 32 and 100 mg/L. GC-FID analysis of the fresh media test samples throughout the test showed measured concentrations of n-hexane to range from less than the LOQ (0.0025 mg/L) to 0.20 mg/L, n-heptane to range from less than the LOQ (0.0026 mg/L) to 0.140 mg/L, n-octane to range from less than the LOQ (0.0026 mg/L) to 0.0811 mg/L and n-nonane to range from less than the LOQ (0.0026 mg/L) to 0.0216 mg/L. Chemical analysis of the old media test samples throughout the test showed measured concentrations to be below or close to the LOQ for the representative n-alkanes. To prepare the test solutions, amounts of test item (23, 73.6, 230, 736 and 2300 mg) were each separately added to the surface of 23 liters of dechlorinated tap water in a mixing vessel with minimal headspace to give the 1.0, 3.2, 10, 32 and 100 mg/L loading rates respectively. After the addition of the test item, the dechlorinated tap water was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a dimple at the water surface. The stirring was stopped after 23 hours and the mixtures allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm was submerged into the vessels, sealed end down, to a depth of approximately 5 cm from the bottom of the vessels. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The WAFs were removed by mid-depth siphoning to give the 1.0, 3.2, 10, 32, and 100 mg/L loading rate WAFs. Over the course of the study, the pH ranged from 6.0-8.6, the temperature ranged from 23-26°C, the dissolved oxygen ranged from 4.6-10.3 mg/L, and the water hardness ranged from 134-158 mg/L $CaCO_3$.

No significant mortalities or sub-lethal effects were noted at 1.0, 3.2, 10, 32 and 100 mg/L loading rate WAF. Survival and growth of *P. promelas* were not significantly reduced at any concentration tested. Based on the nominal loading rates, the 33-day NOEL and LOEL values were 100 and > 100 mg/L loading rate WAF, respectively.
33-day NOEL = 100 mg/L loading rate (WAF)
33-day LOEL > 100 mg/L loading rate (WAF)

Study 2:
The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.
Chemical Name: GTL diesel (Distillates (Fischer-Tropsch), C8-26-branched and linear)
[redacted]

Harlan Laboratories Ltd conducted an early-life stage toxicity test in fathead minnow (*Pimephales promelas)* with an analog test substance (purity not provided) under semi-static conditions with bi-weekly renewal. This study was reported to follow OECD test guideline 210 "Fish, Early-Life Stage Toxicity Test" (1992), and OPPTS 850.1400. Two replicates of 30 eggs were exposed to the control (dechlorinated tap water) or to the test substance prepared as a Water Accommodated Fraction (WAF) at nominal loading rates of 10, 32 and 100 mg/L. Total organic carbon (TOC) analysis of the test preparations showed no significant differences in the amount of carbon present within the 10, 32 and 100 mg/L loading rate WAF test vessels when compared to the control vessels. Given the background level of carbon in the control vessels and also the low level of carbon in the test vessels, it was considered that the majority of the results were around the limit of quantitation of the analytical method. To prepare the test solutions, amounts of test material (110, 352 and 1100 mg) were each separately added to the surface of 11 litres of dechlorinated tap water to give the 10, 32 and 100 mg/L loading rates respectively. The stirring vessels were sealed with minimal headspace to reduce losses due to the possible volatile nature of the test material. After the addition of the test material, the dechlorinated tap water was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a dimple at the water surface. The stirring was stopped after 71 hours and the mixtures allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The WAF was removed by mid-depth siphoning to give the 10, 32, and 100 mg/L loading rate WAFs. Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test material to be present. Over the course of the study, the pH ranged from 6.7-8.6, the temperature ranged from 24-27°C, the dissolved oxygen ranged from 4.4-9.5 mg/L, and the water hardness was 132-144 mg/L $CaCO_3$. No significant mortalities or sub-lethal effects were noted at 10, 32 and 100 mg/L loading rate WAF. The mean hatching rate ranged from 92-95% and the mean survival rate ranged from 93-98%. No significant reductions in fish length or dry weight were observed between the control test groups. Based on the nominal loading rates, the 33-day NOEL and LOEL values were 100 and > 100 mg/L loading rate WAF, respectively.

33-day NOEL = 100 mg/L loading rate (WAF)
33-day LOEL > 100 mg/L loading rate (WAF)

Daphnid Ecotoxicity Tests:

Study 1:
The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.
Chemical Name: GTL Naphtha ((Fischer-Tropsch), light, C4-10-branched and linear)
[redacted]

Harlan Laboratories Ltd conducted a 21-day reproductive toxicity test in daphnids (*Daphnia magna)* with an analog test substance (100% purity) under semi-static conditions with renewal three times per week. This study was reported to follow OECD test guideline 211 "Daphnia magna, Reproduction Test" (2008). Based on the results of an acute toxicity study, ten replicates of a single daphnid were exposed to control (Elendt M7 medium) or to the test substance prepared as a Water Accommodated Fraction (WAF) at nominal loading rates of 0.22, 0.70, 2.2, 7.0, and 22 mg/L. GC-FID analysis of the fresh media showed measured concentrations to range from < LOQ – 0.0695 mg/L for n-hexane and n-heptane, < LOQ – 0.0367 mg/L for n-octane and < LOQ – 0.00372 mg/L for n-nonane. Analysis of old media showed measured concentrations to range from < LOQ – 0.00392 mg/L for n-hexane, < LOQ – 0.00669 mg/L for n-heptane, < LOQ – 0.00723 mg/L for n-octane and < LOQ for n-nonane. The limit of quantitation was determined to be 0.0097 mg/L for n-hexane, 0.0095 mg/L for n-heptane, 0.011 mg/L for n-octane and 0.0098 mg/L for n-nonane for days 0 to 2 and due to a change in instrument sensitivity to be 0.0030 mg/L for n-hexane, 0.0030 mg/L for n-heptane, 0.0029 mg/L for n-octane and 0.0029 mg/L for n-nonane for days 5 to 21.

To prepare the test solutions, amounts of test item (15.75, 49.5, 157.5, and 495 mg) were each separately added, via a plastic syringe, to the surface of 22.5 liters of reconstituted water in separate stirring vessels with minimal headspace to give the 0.70, 2.2, 7.0 and 22 mg/L loading rates. A further amount of test item (4.95 mg) was added to a glass slide and suspended in the water column of 22.5 litres of reconstituted water in a stirring vessel with minimal headspace to give the 0.22 mg/L loading rate. After the addition of the test item, the reconstituted water was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a slight dimple at the water surface. The stirring was stopped after 23 hours and the mixtures allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Parafilm was submerged into each vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Parafilm seal. The WAFs were removed by mid-depth siphoning to give the 0.22, 0.70, 2.2, 7.0, and 22 mg/L loading rate WAFs. Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test material to be present. After siphoning and throughout the duration of the test, the loading rate WAFs were observed to be clear, colorless solutions.

Over the course of the study, the pH ranged from 7.3-8.2, the temperature ranged from 21-22°C, the dissolved oxygen ranged from 7.4-9.7 mg/L, and the water hardness was

232-300 mg/L $CaCO_3$. No statistically significant differences in mortalities and sub-lethal effects (i.e. number of live young produced per adult and length of daphnids) were observed in the parental generations exposed to the test substance, compared to the control. No effects on the filial generation were observed in the test replicates; the numbers of unhatched eggs and dead young were low in all control and treatment groups surviving to maturation. Based on nominal loading rates, the 21-day EL_{50} values for immobilization and reproduction were greater than 22 mg/L loading rate WAF. The 21-day LOEL and NOEL were 22 and > 22 mg/L loading rate WAF, respectively.

21-day EL_{50} (immobilization and reproduction) > 22 mg/L loading rate WAF
21-day NOEL (immobilization and reproduction) = 22 mg/L loading rate WAF
21-day LOEL (immobilization and reproduction) > 22 mg/L loading rate WAF

Study 2:

The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.

Chemical Name: GTL diesel (Distillates (Fischer-Tropsch), C8-26-branched and linear)

[redacted]

Harlan Laboratories Ltd conducted a 21-day reproductive toxicity test in daphnids (*Daphnia magna)* with an analog test substance (purity not provided) under semi-static conditions with renewal three times per week. This study was reported to follow OECD test guideline 211 "Daphnia magna, Reproduction Test" (1998). Ten replicates of a single daphnid were exposed to the control (dechlorinated tap water) or to the test substance prepared as a Water Accommodated Fraction (WAF) at nominal loading rates of 10, 32 and 100 mg/L. Total organic carbon (TOC) analysis of freshly prepared solutions showed the amount of carbon present within the test vessels to range from < LOQ – 4.30 mg C/L (LOQ = 1.0 mg C/L). On days 0, 5 and 16 the TOC analysis was slightly high: 5.45 – 10.84 mg C/L in test vessels and 1.93 – 5.20 mg C/L in controls. Corresponding frozen duplicate samples ranged from 2.17 – 5.27 mg C/L in test vessels and 1.59 – 4.26 mg C/L in control vessels; therefore, the slightly high values were attributed to carry over from other samples during analysis. Due to the elevated TOC levels observed in the definitive test, an additional experiment was conducted using a single loading rate of 100 mg/L and a control; this test was conducted following the same procedures used in the definitive test. In the additional test, TOC ranged from < LOQ – 3.86 mg C/L in new media and < LOQ – 1.90 mg C/L in old media.

To prepare the test solutions, amounts of test item (110, 352, and 1100 mg) were each separately added to the surface of 11 liters of dechlorinated tap water to give the 10, 32 and 100 mg/L loading rates respectively. The stirring vessels were sealed with minimal headspace to reduce losses due to the possible volatile nature of the test item. After the addition of the test item, the dechlorinated tap water was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a dimple at the water surface. The stirring was stopped after 71 hours and the mixtures allowed to stand for 1 hour. Visual inspection of 10, 32 and 100 mg/L WAFs showed cloudy water columns and hence it was considered justifiable to remove the WAFs by filtering through a glass wool plug (2-4 cm in length). A wide bore glass tube, covered at one end with Parafilm was submerged into

the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Parafilm seal. A glass wool plug was inserted into the opposite end of the tubing and the WAF removed by mid-depth siphoning to give the 10, 32 and 100 mg/L loading rate WAFs. Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test material to be present. The freshly prepared loading rate WAFs were observed to be clear, colorless solutions whilst the old media were observed to be pale green dispersions due to the presence of algal cells used as feed for the daphnids.

Over the course of the study, the pH ranged from 7.4-8.1, the temperature ranged from 19-21°C, the dissolved oxygen ranged from 6.5-9.5 mg/L, and the water hardness was 140-170 mg/L $CaCO_3$. The number of mortalities in the parental generation was significantly increased only at 100 mg/L loading rate WAF, which showed 100% mortality. No statistically significant differences in sub-lethal effects (i.e. number of live young produced per adult and length of daphnids) were observed in the parental generations exposed at 10 and 32 mg/L loading rate WAFs, compared to the control. No effects on the filial generation were observed at 10 and 32 mg/L loading rate WAFs; the numbers of unhatched eggs and dead young were low in all control and treatment groups surviving to maturation.

In the additional experiment, temperature was maintained at approximately 20°C, air saturation values (ASV) ranged from 68-121% and water hardness ranged from 114-176 mg/L $CaCO_3$. No significant immobilization was observed at 100 mg/L loading rate WAF; however, significantly fewer young were produced and the adult daphnids were observed to be pale and significantly smaller than those in the control. Based on nominal loading rates, the 21-day EL_{50} values for immobilization and reproduction were 57 mg/L loading rate WAF. The 21-day LOEL and NOEL values were 100 and 32 mg/L loading rate WAF, respectively.

21-day EL_{50} (immobilization and reproduction) = 57 mg/L loading rate WAF
21-day NOEL (immobilization and reproduction) = 32 mg/L loading rate WAF
21-day LOEL (immobilization and reproduction) = 100 mg/L loading rate WAF

Study 3:

The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.

Chemical Name: Kerosine (Fischer-Tropsch), narrow cut, C 8-13 – branched and linear

[redacted]

Harlan Laboratories Ltd. conducted a 21-day reproductive test in daphnids (*Daphnia magna*) with an analog test substance (purity not provided; complex mixture) under semi-static conditions with renewal three times per week. This study was reported to follow OECD test guideline 211 "Daphnia magna, Reproduction Test" (2008). Ten replicates of a single daphnid were exposed to a dilution water control (Elendt M7 medium) or the test substance prepared as a Water Accommodated Fraction (WAF) at a nominal loading rate of 1.0, 3.2, 10, 32 or 100 mg/L. Chemical analysis (GC-FID) of the fresh and old media test samples throughout the test showed measured concentrations to be below or close to

the limit of quantitation for the representative n-alkanes (assessed as 0.0018 mg/L for n-heptane and n-undecane, 0.0020 mg/L for n-octane, 0.0019 mg/L for n-nonane and n-decane and 0.0016 mg/L for n-dodecane, n-tridecane and n-tetradecane). Measured concentrations were obtained for day 12 (old and fresh media). These results were considered to be due to post sampling contamination given that the analysis showed similar measured concentrations regardless of initial loading rate or n-alkane.

To prepare the desired nominal loading rates, appropriate amounts of test item were each separately added, via a plastic syringe, to the surface of 11 liters of reconstituted water in a mixing vessel with minimal headspace. After the addition of the test item, the reconstituted water was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a slight dimple at the water surface. The stirring was stopped after 23 hours and the mixtures were allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm, was submerged into each vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Necofilm seal. Microscopic inspection of the WAFs showed no micro-particles of test item to be present. The aqueous phases or WAFs were removed by mid-depth siphoning (the first 75-100 mL discarded) to give the desired loading rate WAFs. After siphoning and for the duration of the test, the test solutions were observed to be clear, colorless solutions.

Over the course of the study, temperature ranged from 21 – 22°C, pH ranged from 7.9 – 8.3 and dissolved oxygen ranged from 8.3 – 9.6 mg/L. The dilution water hardness ranged from 248 – 396 mg $CaCO_3$/L. Mortality was observed at all loading rates; however, the observed mortalities in test groups were not significantly different when compared to the control group. The 21-day EL_{50} for immobilization was > 100 mg/L loading rate WAF. The number of live young produced per adult by day 21 was 110, 97, 95, 96, 96 and 88 at 0 (control), 1.0, 3.2, 10, 32 and 100 mg/L loading rate WAF; significant differences were found between the control and the 100 mg/L loading rate WAF test group. The 21-day EL_{50} for reproduction was > 100 mg/L loading rate WAF. A statistically significant difference in terms of daphnid length was observed between all loading rates > 1.0 mg/L compared to the control. A review of the data indicated that this was probably due to a small number of larger daphnids in the control group, which skewed the data and was not a true reflection of any effect of the test item on parental growth. No unhatched eggs or dead young were observed in the control and treatment groups surviving to maturation. The LOEL was 100 mg/L loading rate WAF as this test group produced significantly fewer live young per adult than the control group; the NOEL was 32 mg/L loading rate WAF.

21-day EL_{50} (immobilization and reproduction) > 100 mg/L loading rate WAF
21-day NOEL = 32 mg/L loading rate WAF
21-day LOEL = 100 mg/L loading rate WAF

Algal Ecotoxicity Tests:

Study 1:

The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.

Chemical Name: Drilling Fluid Narrow Cut (C11-24, branched and linear)
CAS RN: Not provided

Harlan Laboratories Ltd. conducted a 72-hour growth inhibition test in marine algae (*Skeletonema costatum*) with an analog test substance under static conditions. This study was reported to follow ISO Guideline No. 10253 'Water Quality Marine Algal Growth Inhibition Test with *Skeletonema costatum* and *Phaeodactylum tricornutum*' and the OSPAR Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Off-Shore (reference number 2002-3, amended March 2005). Following a range-finding test, six replicates of *S. costatum* (3×10^3 cells/mL) were exposed to a culture medium control or the test substance prepared as a Water Accommodated Fraction (WAF) at a nominal loading rate of 100 mg/L. Chemical analysis of the test loading rates was not conducted. The algae were illuminated at a light intensity of approximately 7000 lux with constant shaking. To prepare the 100 mg/L loading rate, an amount of test material (200 mg) was added to the surface of 2 liters of culture medium using a plastic disposable syringe. After the addition of the test material, the culture medium was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a slight dimple at the water surface. The stirring was stopped after 23 hours and the mixture was allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm, was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or WAF was removed by mid-depth siphoning (the first approximately 75 – 100 mL discarded) to give the 100 mg/L loading rate WAF. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. Test flasks were plugged with polyurethane foam bungs. At the start of the test, all control and test cultures were observed to be clear colorless solutions. After the 72-hour test period, all control and test cultures were observed to be pale brown dispersions. Over the course of testing, temperature was maintained at 24 ± 1°C and pH ranged from 7.9 – 8.1. The mean cell density of control cultures was increased by a factor of 217 after 72 hours. Based on nominal loading rates, the 72-hour EL_{50} was > 100 mg/L loading rate WAF for growth rate and biomass integral. The 72-hour NOEL and LOEL values were 100 and > 100 mg/L loading rate WAF, respectively, for growth rate and biomass integral.

72-hour EL_{50} (growth rate and biomass integral) > 100 mg/L loading rate WAF
72-hour NOEL (growth rate and biomass integral) = 100 mg/L loading rate WAF
72-hour LOEL (growth rate and biomass integral) > 100 mg/L loading rate WAF

Study 2:

The test substance information and study summary below is for an analogous chemical, not the actual PMN substance.

Chemical Name: Drilling Fluid Wide Cut (C9-29, branched and linear)
CAS RN: Not provided

Harlan Laboratories Ltd. conducted a 72-hour growth inhibition test in marine algae (*Skeletonema costatum*) with an analog test substance under static conditions. This study was reported to follow ISO Guideline No. 10253 'Water Quality Marine Algal Growth Inhibition Test with *Skeletonema costatum* and *Phaeodactylum tricornutum*' and the OSPAR Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Off-Shore (reference number 2002-3, amended March 2005). Following a range-finding test, six replicates of *S. costatum* were exposed to a culture medium control or the test substance prepared as a Water Accommodated Fraction (WAF) at a nominal loading rate of 100 mg/L. Chemical analysis of test loading rates was not conducted. The algae were illuminated at a light intensity of approximately 7000 lux with constant shaking. To prepare the 100 mg/L loading rate, an amount of test material (200 mg) was added to the surface of 2 liters of culture medium. After the addition of the test material, the culture medium was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a slight dimple at the water surface. The stirring was stopped after 23 hours and the mixture allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm, was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or WAF was removed by mid-depth siphoning (the first 75 – 100 mL discarded) to give the 100 mg/L loading rate WAF. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test material to be present. Test flasks were plugged with polyurethane foam bungs. At the start of the test, all control and test cultures were observed to be clear colorless solutions. After the 72-hour test period, all control and test cultures were observed to be very pale brown dispersions. Over the course of testing, temperature was maintained at 20 ± 1°C and pH ranged from 8.0 – 8.3. The mean cell density in control cultures were increased by a factor of 216 after 72 hours. The 72-hour EL_{50} was > 100 mg/L loading rate WAF for growth rate and biomass integral. The 72-hour NOEL and LOEL values were 100 and > 100 mg/L loading rate WAF, respectively, for growth rate and biomass integral.

72-hour EL_{50} (growth rate and biomass integral) > 100 mg/L loading rate WAF
72-hour NOEL (growth rate and biomass integral) = 100 mg/L loading rate WAF
72-hour LOEL (growth rate and biomass integral) > 100 mg/L loading rate WAF

APPENDIX 2

The following studies on the 2 PMN cases have been reviewed and are considered to be inadequate by EPA. The study summaries are included in this report for information purposes only.

EPA reviewed the studies below, provided by the sponsor, and has concluded that they are inadequate for the purposes of this PMN review, for the following reasons: 1) The substances tested were of unknown composition - the test substances' purity is not provided and they are described as complex mixtures, 2) the studies used the Water Accommodated Fraction (WAF) method using nominal loading rates with only information of that "at 0 and 72 hours showed measured test concentrations of less than the limit of quantification" – it is not known what chemicals and quantities were actually tested in the WAF testing system.

Algal Ecotoxicity Tests:

Study 3:
Chemical Name: Alkanes, C8-11-branched and linear [redacted]
Trade Name: Shell GTL Solvent GS160
[redacted]

Harlan Laboratories Ltd. conducted a 72-hour growth inhibition test in green algae (*Pseudokirchneriella subcapitata*) with P-14-0132 (purity not provided; complex mixture) under static conditions. This study was reported to follow OECD Guidelines for Testing of Chemicals (2006) No 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" referenced as Method C.3 of Commission Regulation (EC) 761/2009. Following a range-finding test, six replicates of *P. subcapitata* (5×10^3 cells/mL) were exposed to a culture medium control or the test substance prepared as a Water Accommodated Fraction (WAF) at a nominal loading rate of 100 mg/L. Chemical analysis (GC-FID) of the 100 mg/L test preparation at 0 and 72 hours showed measured test concentrations of less than the limit of quantification (LOQ = 0.0902 mg/L). The algae were illuminated at a light intensity of approximately 7000 lux with constant shaking. To prepare the 100 mg/L loading rate, an amount of test item (555 mg) was added to the surface of 5.55 liters of culture medium in a sealed vessel with minimal headspace. After the addition of test item, the culture medium was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a dimple at the water surface. The stirring was stopped after 23 hours and the mixture allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm, was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or WAF was removed by mid-depth siphoning (the first 75 – 100 mL discarded) to give the 100 mg/L loading rate WAF. Microscopic inspection of the WAF showed no micro-dispersions or undissolved test item to be present. Test flasks were sealed with glass stoppers. At the start of the test, all control and test cultures were observed to be clear colorless solutions. After the 72-hour test

period, all control and test cultures were observed to be green dispersions. Over the course of testing, temperature was maintained at 24 ± 1°C and pH ranged from 7.7 – 9.8. The mean cell density of control cultures was increased by a factor of 91within 72 hours. Based on nominal loading rates, the 72-hour EL_{50}, for yield and growth rate, was greater than 100 mg/L loading rate WAF. The NOEL and LOEL values were 100 and > 100 mg/L loading rate WAF, respectively, for growth rate and yield.

72-hour EL_{50} (growth rate and yield) > 100 mg/L loading rate WAF
72-hour NOEL (growth rate and yield) = 100 mg/L loading rate WAF
72-hour LOEL (growth rate and yield) > 100 mg/L loading rate WAF

Study 4:
Chemical Name: Alkanes, C9-12-branched and linear [redacted]
Trade Name: Shell GTL Solvent GS170
[redacted]

Harlan Laboratories Ltd. conducted a 72-hour growth inhibition test in green algae (*Pseudokirchneriella subcapitata*) with P-14-0133 (purity not provided; complex mixture) under static conditions. This study was reported to follow OECD Guidelines for Testing of Chemicals (2006) No 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" referenced as Method C.3 of Commission Regulation (EC) 761/2009. Following a range-finding test, three replicates of *P. subcapitata* (5×10^3 cells/mL) were exposed to the test substance prepared as a Water Accommodated Fraction at nominal loading rates of 10, 25, 62.5, 156 or 391 mg/L. Six replicates of *P. subcapitata* were exposed to a culture medium control. Chemical analysis (GC-FID) of the 391 mg/L loading rate WAF test preparations at 0 and 72 hours showed measured test concentrations of less than the limit of quantification (LOQ = 0.22 mg/L). The algae were illuminated at a light intensity of approximately 7000 lux with constant shaking. To achieve the desired loading rates, amounts of test item were each separately added to the surface of 5.5 liters of culture medium in sealed vessels with minimal headspace. After the addition of the test item, the culture medium was stirred by magnetic stirrer using a stirring rate such that a vortex was formed to give a dimple at the water surface. The stirring was stopped after 23 hours and the mixtures allowed to stand for 1 hour. A wide bore glass tube, covered at one end with Nescofilm, was submerged into the vessel, sealed end down, to a depth of approximately 5 cm from the bottom of the vessel. A length of Tygon tubing was inserted into the glass tube and pushed through the Nescofilm seal. The aqueous phase or WAF was removed by mid-depth siphoning (the first 75-100 mL discarded) to give the desired loading rate WAFs. Microscopic inspection of the WAFs showed no micro-dispersions or undissolved test item to be present. Test flasks were sealed with glass stoppers. At the start of the test, all control and test cultures were observed to be clear colorless solutions. After the 72-hour test period, all control and test cultures were observed to be green dispersions. Over the course of testing, temperature was maintained at 24 ± 1°C and pH ranged from 7.9 – 9.7. The mean cell density of control cultures was increased by a factor of 71 within 72 hours. Based on nominal loading rates, the 72-hour EL_{50}, for growth rate and yield, was > 391 mg/L loading rate WAF. The 72-hour NOEL and LOEL values were 391 and > 391 mg/L loading rate WAF, respectively, for growth rate and yield.

72-hour EL_{50} (growth rate and yield) > 391 mg/L loading rate WAF
72-hour NOEL (growth rate and yield) = 391 mg/L loading rate WAF
72-hour LOEL (growth rate and yield) > 391 mg/L loading rate WAF